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Fort Detrick, Maryland

The Importance of the Plague Microbe's Capsule in the Problem of Live Vaccines.

by A. D. Garmazova

Translated form Izvestiya Irkutskogo Gosudarstvennogo Nauchno-Issledovatel'skogo Protivochumnogo Instituta Sibiri i Dal'nego Vostoka (Reports of the Irkutsk State Scientific-Research Antiplague Institute of Siberia and the Far East), v 20, 1959, p 199-206, by SFC Eldon E. Ewing, Technical Library, Technical Information Division.

The development of highly effective plague vaccines is a question of primary importance in the prophylaxis of plague.

The killed plague vaccines have not proven themselves in practice in view of their slight efficacy. The advent of the live plague vaccines was an important step forward in this regard.

The important problem in the development of highly effective live plague vaccines is the detailed study of the antigenic structure of the plague microbe, and the explanation of the role of each component of the microbic cell in immunogenesis.

In recent times, articles have been appearing more and more frequently in the literature, stating the importance of the plague microbe's capsule in creating immunity to plague. Several of the available works experimentally prove this position (Korobkov, Korobkova and Bakhrakh, Zheltenkov and Anokhina, Zhukov-Verezhnikov, Shyuttse et al).

The cited authors demonstrate the superiority of the capsular plague cultures, in an immunological sense, over the noncapsular, both in live as well as in killed vaccines.

Thus, Shyuttse considers that plague cultures grown at 37°C (having capsules) transmit a more persistent immunity to white rats (72% survive after inoculation) than does the same culture grown at 25°C (noncapsular), where 36% survive.

Karauchi and Homma prepared a vaccine from an isolated capsular substance of the plague microbe and found it more effective than usual bacterial vaccines. They believe that a single injection of this substance (20 units) in a subcutaneous administration makes a human immune to plague.

Korobkova, Favorisova and Kolesnikova investigated the efficacy of killed AD vaccines on guinea pigs. The vaccines were prepared from virulent plague cultures grown at 37°C and at 28°C. The guinea pigs, which were immunized subcutaneously in three stages and challenged with 5 lethal doses of a virulent strain 14 days after immunization, had a survival rate of 39% in the first instance and a total of 4% in the latter.

These same authors compared the immunogenic properties of Kolle's killed vaccine that was prepared from a virulent culture of the plague microbe grown at 37°C and at 28°C. In a challenge with 5 lethal doses, 8% of the guinea pigs immunized three times with the first type of vaccine survived, while all of those immunized with the second vaccine died.

In the recent works of Korobkova (1951) and Zheltenkov, in conjunction with Anokhina (1951), the superiority of the capsular live vaccines EV as compared to the noncapsular was also demonstrated.

In the experiments of Zheltenkov and Anokhina, white mice were immunized three or five times with subcutaneous injections of the EV culture grown at 37°C and at 28°C. After a month, the mice were inoculated with massive doses of a virulent strain (100, 250, 500 and 1,000 Dcl). The mice immunized with the first type of vaccine had a survival rate of 28.5% to 100%; in the second group, the survival rate was expressed at 11.1%-66.7%.

Korobkova immunized guinea pigs with an EV culture grown on a special medium that she developed (which gave an increased capsulation of the plague strains) and with an EV culture grown on a common medium. A dose of 1 billion microbic bodies of the first culture, given subcutaneously, conferred immunity on 86.7% of the guinea pigs that were challenged with 200 Dcl 22 days after immunization. A total of 60% of the animals in the second group survived this same dose.

All of this indicates that both the killed and the live plague vaccines that are prepared from capsular cultures are more full-valued than those prepared from the noncapsular cultures.

Soviet investigators have also proven the superiority of the capsular forms of plague cultures in the practice of obtaining more effective anti-plague sera (Zhukov-Verezhnikov).

On this question, however, there are also different viewpoints in the literature. Thus, Otten found no differences in the immunogenic properties of plague cultures grown at 37°C and at 28°C. Sokhey and Moris believe that the plague cultures grown at 27°C are more immunogenic than the same cultures grown at 37°C.

The present work is dedicated to a study of the rationality of the development and use of capsular live plague vaccines

Experimental Section

The basis of our work was the comparison, on laboratory animals, of the immunogenic properties of an EV culture having large capsules with an EV culture having small capsules. White mice were used primarily as the experimental animals, with guinea pigs being used in part.

Some of the white mice were immunized with a single injection of the large-capsular EV cultures (10 million microbes subcutaneously) and the others were immunized with the small-capsular variant in the same dosage injected subcutaneously at the root of the tail. The guinea pigs were injected subcutaneously with 1.5 billion microbes in the region of the haunch.

The EV culture with the large capsules was grown under conditions of a high content of carbon dioxide (15-20%) on Marten's agar (pH 7.2) at 37°C for a period of 48 hours (Chertnik, 1940). We produced the necessary concentration of carbon dioxide in an exsiccator, or beneath a glass bell by means of a chemical reaction between a 25-% solution of sulfuric acid and sodium carbonate. All of the plague strains, including the EV, produced broad capsules under these conditions.

We noted one fact that is extremely interesting: that an EV culture grown once in the presence of carbon dioxide, long retains an increased capacity for capsulation under normal conditions. We transplanted an EV strain with the large capsules onto plain agar for a period of 3 months (for a course of 39 generations) and during all of this time it retained its ability for profuse capsulation.

Left for 11 days at room temperature (20°C) without transplanting, the strain lost this ability.

We grew the small-capsular cultures on Marten's agar (pH 7.2) at 20°C in a normal atmosphere. When grown under these conditions, the plague bacillus has a capsule of insignificant dimensions.

We were unsuccessful in obtaining a non-capsular culture. Shaking in a shaking machine for 3-4 hours did not completely liberate the microbe from the capsule.

The first part of the work was devoted to an investigation of the relative characteristics of the immunity created by the large-capsular and small-capsular EV cultures in white mice and guinea pigs. There were 5 experiments conducted on the mice, with a total number of 181 animals. The mice were given a single subcutaneous injection of 10 million microbes of the large-capsular or small-capsular vaccines. After 15 days, 21 days, 40 days and 41 days after immunization, the mice were challenged with 100 and 200 lethal doses of a virulent strain of the plague microbe. As a result of the inoculation, the survival rate among the mice immunized with the large-capsular EV culture equalled 42%-100%; the mice immunized with the small-capsular culture had a survival rate of 9%-42% (see table 1). As a rule, the mice of the first group lived longer than the control and the mice immunized with the small-capsular EV culture.

There were 2 experiments conducted on the guinea pigs (37 g. pigs). The animals were immunized subcutaneously with a single dose of 1.5 billion microbes. Within 32 days and 41 days after immunization the animals were inoculated with 100 Dcl, 200 Dcl and 500 Dcl. As a result of the inoculation, all of the guinea pigs immunized with the large-capsula· EV culture survived; in the group of guinea pigs vaccinated with the small-capsular EV culture, the survival rate equalled 60%-80%, with a 100% death of the control animals (see table 1).

We conducted 3 experiments on white mice (217 mice) to resolve the question concerning the intensity of the immunity created by the large-capsular and small-capsular EV cultures. The immunization was made in a single injection by our usual method (10 million microbes subcutaneously). The animals were inoculated with several increasing doses (100 Dcl, 500 Dcl, 1,000 Dcl, 2500 Dcl and 10,000 Dcl) of a virulent strain of the plague microbe after 22 days, 33 days and 41 days from the time of immunization (see table 2). The experiments show the superiority of the large-capsular cultures over the small-capsular with complete clarity. The immunity created by the former is 3-4 times more intense than that produced by the small-capsular culture: 70%-54% of the mice withstand such massive doses of a virulent culture as 2500 and 10,000 Dcl, whereas the mice of the second group give a survival rate of 0 to 18%.

The question concerning the times of occurrence and the dynamics of growth of immunity in laboratory animals when vaccinated with live plague vaccines is an important, but inadequately investigated subject. We resolved these questions in regards to the large-capsular and small-capsular vaccines on white mice.

There were 2 experiments conducted, with a total number of 325 mice. The mice were subjected to a single subcutaneous immunization with 10 million microbes. After 1 day, 2 days, 3 days, 4 days, 5 days,6 days, 8 days, 13 days and 20 days from the time of immunization, the mice were inoculated with a virulent culture (100 Dcl). The results of the inoculation show that by the end of 6-8 days, 60%-70% of the mice vaccinated with the large-capsular culture obtain an insusceptibility to plague. In the group of mice immunized with the small-capsular EV vaccine, the survival rate reached 20%-30% by the end of 6-8 days and is preserved at this level in all of the groups for 20 days (the time of observation).

The first signs of immunity appear significantly earlier: within 24 hours, 30%-40% of the mice survive 100 Dcl. Towards the end of the second day the sensitivity of the mice rises (10% of the mice survive). The fourth day gives a new rise in the insusceptibility, when 60% of the first group and 20% of the second group survive. The fifth day is marked by a new drop of immunity (20% survive in the first group; 10% in the second). The times of the mice's survival after inoculation were also shortened in accordance with this.

Thus, during the first 6 days after immunization, we observe an alternation of an immune condition with periods of increased susceptibility to plague. From the 6th to the 20th day, the immunity remains at one level or is somewhat raised (60%-70% in the first; 20% in the latter; see table 3).

Experiments were conducted on white mice and guinea pigs to determine the duration of the immunity created by the subject cultures.

The white mice were given a single subcutaneous injection of 10 million microbes from the large-capsular or the small-capsular EV cultures. Of the mice immunized with the large-capsularculture, with an inoculation of 200 Dcl after 1.5 months, 70% survived; of those immunized with the small-capsular vaccine, 60% survived; with an inoculation of 100 Dcl after 3 months, 63.3% survived in the first group, and 44.5% in the second; an inoculation with 100 lethal doses made 4 months after immunization showed a complete lack of immunity in both groups of the mice. The mice died at the same times as the control. An inoculation of 50 Dcl at this same time gave a survival rate of 58.4% in the first mice and 30% in the second.

The guinea pigs sere given a single subcutaneous injection of 1.5 billion microbes from the large-capsular or small-capsular EV culture. After 2 months, 70% of the first group survived a challenge of 100 Dcl, and 30% of the guinea pigs in the second group survived; with a challenge of 50 Dcl after 4 months, the first group had a 90-% survival and the second had 50%.

Conclusions

1. Growing plague cultures, including the EV strain, on common media in an atmosphere with an increased carbon dioxide content at 37°C contributes to their intensified capsulation.

2. An EV strain grown in the presence of carbon dioxide, long retains the increased capsulation capacity under normal conditions.

3. An EV culture grown under the conditions of an increased content of carbon dioxide at 37°C,and having broad capsular dimensions, possesses a greater immunogenic capacity than the same culture grown under ordinary conditions at 20°C and having insignificant capsular dimensions.

4. A single vaccination with the large-capsular culture (10 million microbes subcutaneously) transmits to white mice a 75%-100% immunity to 100 lethal doses, and higher, of a virulent strain of the plague microbe, whereas its small-capsular variant, with the same immunizing dose, can protect only 20%-50% of the mice from death.

5. The immunity produced by the large-capsular EV culture is more intense than that created by the small-capsular variant of this strain. A single immunization with this culture (10 million microbes subcutaneously) protects 70%-54.6% of the white mice from such massive doses ofa virulent strain as 2500-10,000 Dcl, with the challenge 41 days after immunization. The small-capsular EV culture protects only 10%-18.2% from these doses.

6. The first signs of plague immunity of the mice immunized with the EV culture appear within 24 hours (30%-40% of the mice survive 100 Dcl). During the following 6 days the immunity, in the process of growth, gives

a series of alternations of some immunity (4th and 6th days) with periods of increased susceptibility to the plague infection (2nd and 5th days). Later, the immunity continues to grow without noticeable fluctuations.

7. A large survival rate is observed in the animals immunized with the EV culture containing the large capsules both in the early periods after immunization (the first 4 days) as well as in the later periods (4 months).

8. The great efficacy of the EV cultures with the large capsules is also shown in the experiments on the guinea pigs. This culture, in a dose of 1.5 billion microbes injected subcutaneously, transmits immunity to 100% of the guinea pigs challenged with 100, 200 and 500 lethal doses of a virulent strain. The guinea pigs immunized with the small-capsular EV culture have a survival rate of 60%-80%. In a challenge inoculation of 50 Dcl made 4 months after immunization, the survival rate was 90% in the first group and 50 % in the second.

9. By increasing the capsulation capacity in the EV strain, we improve its immunogenic qualities, which is very important in preparing highly effective live plague vaccines.

Table 3
(Tables 1 & 2 not available to translator)

No. of test	Inoculation dose in Dcl	Times of inoculation after immunization (in days)	Large-Capsule Vac.				Small-Capsule Vac.				Control			
			Mice inoculated	Died	Percentage of survival	Day of death	Mice inoculated	Died	Percentage of survival	Day of death	Mice inoculated	Died	Percentage of survival	Day of death
1	100	1	10	7	30	9.6	10	6	40	7	5	5	-	6
		2	10	9	10	7.7	10	9	10	5	5	5	-	5.2
		3	10	8	20	5	10	9	10	5.7	5	5	-	5
		4	5	2	60	12	15	12	20	10.3	5	5	-	3.6
		5	10	8	20	5.7	10	9	10	6.1	5	5	-	6.8
		8	10	3	70	10	10	8	20	6	5	5	-	6.8
	Total		55	37	32.8	8.3	65	53	18.4	6.7	30	30	-	5.5
2	100	4	10	8	20	4.6	10	8	20	3.7	10	10	-	3
		6	10	4	60	6.7	10	9	10	5.4	5	5	-	5.2
		8	9	2	78	7.2	10	7	30	6.8	5	5	-	4.4
		13	10	2	80	10	8	4	50	7.5	5	5	-	5.2
		20	14	4	71.4	8.7	9	6	33.3	6	10	10	-	4.8
	Total		53	20	62.3	7.4	47	34	27.6	5.8	35	35	-	4.8